A COMPARISON OF MICROBIAL ACTIVITY IN AN ONTARIO FOREST SOIL UNDER PINE, HEMLOCK, AND MAPLE COVER[1]

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Abstract

Soil samples, taken by horizons, were obtained from the University of Toronto Forest near Dorset under stands of maple, hemlock, and pine. Counts of bacteria, actinomycetes, and fungi made by the plate method indicated that in general the organic layer contained the largest population, and also that in the organic layer under conifers the fungi exceeded the combined counts of the other two groups, whereas under maple the bacteria predominated. Using the perfusion method, nitrification did not occur to any extent in these forest soil samples except when lime was added, and even then nitrification started very slowly unless a few crumbs of garden soil were added, presumably as a source of active nitrifying bacteria.

Introduction

The increasing demand for products of our forests has intensified interest in the art of forest management. The realization that regeneration of desirable species is fraught with uncertainty has emphasized the need for a better understanding of those factors which contribute to the success or failure of maintaining selected species in the complex forest environment. One of the environmental factors which has received very little attention, particularly on this continent, is that of the microorganisms in the soil.

Russell recently discussed the characteristics of soils developed under forest conditions, particularly the humus layer (15). He states that mor formation typically occurs under coniferous forest growing on acid soils low in calcium; that in a typical mor, fungi are often considered the most important group (though proof is lacking); and that nitrifying bacteria are typically absent or present in very small numbers, so that mineralized nitrogen tends to appear as ammonia rather than nitrate. In contrast, a mull formation typically develops under deciduous or mixed forests on less acid soils reasonably supplied with calcium. Bacteria are probably the most important microbial agents, and with nitrifying bacteria present inorganic nitrogen is usually found in the form of nitrate rather than ammonia. However, Russell points out that while mor formation typically occurs under coniferous forest, exceptions do occur, and thus mull may be found associated with conifers, and likewise mor sometimes develops under deciduous forest. Thus, while typical associations suggest the general conditions that one may anticipate under coniferous or deciduous forests, since exceptions occur it becomes necessary to investigate conditions in new areas, rather than risk making generalizations.

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This paper presents the results of microbial studies made on soil horizons developed from granitic materials of the Pre-Cambrian shield under different species of tree cover, but with other environmental factors being similar.

Experimental Material

Description of Forest Soil

Soil samples were obtained from the University of Toronto Forest (8) within a five-mile radius of the Forest Rangers' School at Lake St. Nora, which is on Ontario Provincial Highway 35, approximately 18 miles south of the western entrance to Algonquin Park. Three locations were selected, having similar site classifications which include such factors as slope, drainage, exposure, ecoclimate (7), but representing different tree cover. Samples were taken by horizon from a pit at each of the sites chosen, and are described below:

Maple Samples

The maple stand was on somewhat excessively drained sandy loam of the brown podsolic soil type (little or no leached layer). The Pre-Cambrian granite bedrock occurred at a depth of 28–30 in.

Maple A_{01}, A_1.—1 to 3 in. of fibrous mor, fairly well decomposed over a trace to $\frac{1}{4}$ in. of a crumblike layer consisting largely of organic matter but with some mineral.

Maple B_2.—10 to 12 in. of silty sand with humus (not iron) colloids.

Maple B_{3g}.—loamy sand, incipiently gleid, between the B_2 and bedrock.

Hemlock Samples

The hemlock stand was on a physiographic site similar to the maple, except the soil was a weak podsol.

Hemlock A_{01}.—$\frac{3}{4}$ to $1\frac{1}{2}$ in. of fairly well decomposed leaf litter of the fibrous mor type.

Hemlock A_1, A_2.—a trace of melanized humus combined with $\frac{1}{2}$ to 2 in. of leached sandy loam, gray in color, with considerable infiltrated humus.

Hemlock B_2.—4 to 6 in. of silty sand, cinnamon brown in color with some iron and humus colloids.

Pine Samples

The pine stand was on an excessively drained sand of podsol characteristics.

Pine A_{01}.—$\frac{1}{4}$ to $\frac{3}{4}$ in. of fibrous mor, partially decomposed and predominantly of pine needle litter.

Pine A_1, A_2.—a trace of sand combined with a trace to 1 in. of leached sand with considerable infiltrated humus.

Pine B_2.—7 to 8 in. of dark brown sand with iron and humus coating (not colloidal).

Chemical Properties of Soil Samples

As soon as the samples were brought to the laboratory, soil pH was determined using a Beckman pH meter and the paste method of Reed and Cummings (13). A portion of each sample was air-dried, and set aside for the following tests: total nitrogen was measured by the micro-Kjeldahl method; organic matter was measured by the wet cumbustion method of Walkley (20), but with ortho-phenanthroline indicator being substituted for the diphenylamine originally used (6); and total exchange capacity was determined by the method of Schollenberger and Simon (16). Results are presented in Table I, in which the feature of greatest interest is the similarity in soil pH under maple cover to that under the conifers. It is also obvious that both total nitrogen and total exchange capacity are closely associated with the quantity of organic matter.

TABLE I

CHEMICAL CHARACTERISTICS OF THE HORIZONS OF A FOREST SOIL UNDER THREE SPECIES OF COVER

Tree cover	Horizon	pH	Organic matter (%)*	Total nitrogen (%)*	Total exchange capacity (m.e. per 100 gm.)*
Maple	A_{01}, A_1	4.28	23.	0.76	34.3
	B_2	4.75	9.6	0.28	17.8
	B_{3g}	5.35	5.2	0.35	13.2
Hemlock	A_{01}	4.20	65.	1.32	93.7
	A_1, A_2	3.92	1.4	0.22	7.6
	B_2	5.31	6.4	0.13	20.5
Pine	A_{01}	4.49	19.	0.44	68.7
	A_1, A_2	4.55	3.7	0.48	7.6
	B_2	5.45	2.8	0.11	8.1

* *Corrected to oven-dry weight.*

Enumeration of Soil Microorganisms

Studies of microbial populations in European forest soils have revealed a tendency for bacteria to predominate under deciduous forest and fungi under coniferous forest; this is shown in several reviews of the subject (4, 11, 15). Similarly, working with forest soils in New York, Cobb (3) found greater numbers of bacteria and actinomycetes in the topsoil of deciduous forest, while the comparable layer beneath hemlock contained the larger population

of fungi. Since the pH values of these topsoils were similar, there was no apparent relationship between acidity and numbers of the various groups of organisms present.

In the present investigation the plate method was used to obtain an estimate of the aerobic population in the different horizons under the three types of tree cover. Unfortunately the plating could not be made until one week after the samples were taken. Suitable dilutions were used to inoculate triplicate plates of soil extract agar (10), the plates being incubated at 28° C. for one week, at which time bacteria and actinomycetes were counted. From the same dilution series, triplicate plates of rose bengal medium (17) were prepared and from these plates fungi were counted after four days at 28° C.

The results are shown in Table II. The most interesting feature was the preponderance of bacteria in the maple organic layer, almost equal to the actinomycete and fungus counts combined; whereas in the coniferous organic layer, fungus counts exceeded the combined bacterial and actinomycete populations. While this favoring of bacteria by deciduous litter, and fungi by coniferous litter, is in accord with the results of workers elsewhere (3, 4, 15), it is apparent from Table I that these results could hardly be due to a pH effect. The selective action appears to be due to some characteristic of the litter layer other than pH, such as the nature of the organic residue, as suggested by the work of Vandecaveye and Katznelson (18). Except for the relatively large actinomycete populations in the second levels of the maple and hemlock samples, the microbial populations were quite uniform throughout the second and third levels.

TABLE II

PLATE COUNTS OF MICROORGANISMS IN THE HORIZONS OF A SOIL UNDER THREE TYPES OF FOREST

(In hundred thousands per gram oven-dry soil)

Tree cover	Horizon	Bacteria	Actinomycetes	Fungi
Maple	A_{01}, A_1	4.2	2.8	2.6
	B_2	1.0	3.5	1.7
	B_{3g}	0.7	1.0	0.1
Hemlock	A_{01}	3.5	4.6	13.1
	A_1, A_2	0.7	3.4	1.0
	B_2	0.7	0.4	1.0
Pine	A_{01}	2.6	7.4	11.8
	A_1, A_2	1.0	0.7	1.5
	B_2	1.4	2.3	0.1

Nitrifying Capacity

The significance of the nitrification process in forest soils is a topic which does not appear to be well defined. Thus Wilde (21) considers it of importance chiefly when dealing with deciduous lime-loving trees that require nitrogen in the form of nitrates, but not for acidophilous conifers which readily utilize nitrogen as ammonia and possibly amino acids. On the other hand, Lutz and Chandler (11) state that little is known about the nitrogen nutrition of forest trees other than that they depend principally on nitrate and ammoniacal forms. They further conclude that from present evidence there is little reason to maintain that nitrate nitrogen is superior to ammonia nitrogen. It seems that such divergent views could arise according to whether emphasis were placed on the typical associations in mor and mull (see Introduction), which would support the pattern suggested by Wilde, or upon the exceptions, which could lead to the different conclusions reached by Lutz and Chandler.

Since the samples in the present study included the typical combination of mor with conifers, and the less common association of mor with maple, a study of the nitrifying capacities was considered of especial interest.

Methods

The perfusion method of Lees and Quastel (9) was selected to determine the nitrifying capacity of these samples. Perfusion units of the type described by Audus (1) were used. The soil samples were air-dried and sieved to separate the 1 to 4 mm. crumb fraction which was used in the units. Portions of each sieved sample, ranging from 10 to 40 gm. depending upon volume weight, were placed in separate units and perfused with 200 ml., (*a*) of distilled water—to ascertain the nitrification of soil nitrogen, and (*b*) of *N*/50 ammonium sulphate—to determine nitrifying capacity in the presence of added ammonium-nitrogen.

Because of the strong acidity of these soil samples, it was not expected that much nitrification could occur without pH adjustment, though a trial was considered necessary since the limiting pH for nitrification has been reported as low as 3.7 (19) and the samples were all above this. In this first test no nitrate appeared in four weeks, hence a second trial was prepared and calcium carbonate added to the soil in the units in amounts which raised the initial pH to range from 6.2 to 7.5. Table III shows the quantities of soil and lime used; and to give a clearer conception of this lime treatment, its equivalent has been expressed as tons of lime per acre per inch of horizon in the forest. It must be emphasized that accurate volume weight measurements of the horizons were not made, so that the forest requirements are presented as approximations only. The liming treatment was only partially successful, because in 7 out of 36 units the pH of the perfusate dropped to 5.0 or below by the end of the 28-day experimental period. This degree of acidity only reoccurred in units containing added ammonium sulphate. To determine whether the introduction of active nitrifying bacteria would hasten nitrification in these soil samples, paired units of each sample were prepared, and one

TABLE III

LIME TREATMENT OF FOREST SOIL SAMPLES IN PERFUSION UNITS, AND ESTIMATED FOREST EQUIVALENT

Tree cover	Horizon	Amount in each perfusion unit		pH 2 days after lime	Forest equivalent of lime treatment† (tons per acre-inch)
		Soil*, in gm.	Calcium carbonate, in gm.		
Maple	A_{01}, A_1	20	0.60	6.4	2.5
	B_2	40	0.80	7.5	3.0
	B_{3g}	40	0.16	7.0	0.6
Hemlock	A_{01}	10	0.40	6.2	1.7
	A_1, A_2	40	0.20	6.7	0.8
	B_2	40	0.16	6.2	0.7
Pine	A_{01}	10	0.40	6.2	1.7
	A_1, A_2	25	1.00	6.2	4.0
	B_2	40	0.20	6.9	0.8

* *Amounts represent approximately equal volumes, hence indicate ratio of volume weight of samples.*

† *Calculation based on relative volume weights of different profile samples; lowest levels assumed roughly equivalent to agricultural field soil (1.3 gm. per cc.).*

TABLE IV

CHANGES IN pH OF PERFUSATE DURING NITRIFICATION IN LIMED FOREST SOIL WITH AND WITHOUT ADDED NITROGEN

Tree cover	Horizon	Perfused with			
		Water		*N*/50 Ammonium sulphate	
		14 days	28 days	14 days	28 days
Maple	A_{01}, A_1	6.3	5.3	6.6	5.6
	B_2	7.4	7.4	7.5	6.2
	B_{3g}	7.2	6.4	5.4	5.2
Hemlock	A_{01}	6.8	5.8	6.6	6.2
	A_1, A_2	5.6	5.2	6.6	4.6
	B_2	6.0	5.7	5.0	4.6
Pine	A_{01}	6.5	6.3	6.6	5.0
	A_1, A_2	7.4	7.8	6.9	6.8
	B_2	6.6	6.0	5.3	4.8

unit of each pair was inoculated by placing approximately 0.1 gm. of fresh garden soil on the surface of the forest soil in the sample tube.

Progress of nitrification was followed at three- to four-day intervals by estimating nitrate-nitrogen using the method of perfusate analysis described by Chase (2), but with the amount of hydrogen peroxide used to oxidize the

organic matter increased (to 0.5 ml. of 10% hydrogen peroxide) for the samples containing high concentrations of organic matter. Nitrite-nitrogen in the perfusate was determined using the method of Rider and Mellon (14). Seldom did more than a trace of nitrite appear in the distilled water perfusate; and though nitrite often accumulated for a time in the ammonium sulphate perfusate, this did not alter the general trends found in the nitrate data, and hence only the latter are reported in this paper. The pH of the perfusate was measured with a Beckman pH meter each time other analyses were made.

Results

The lime treatment and pH of the perfusates at the middle and end of the 28-day period are presented in Table IV (the pH two days after liming was included in Table III). The results indicate the heavy lime applications necessary to adjust the pH of these acid soils close to neutrality, and maintain

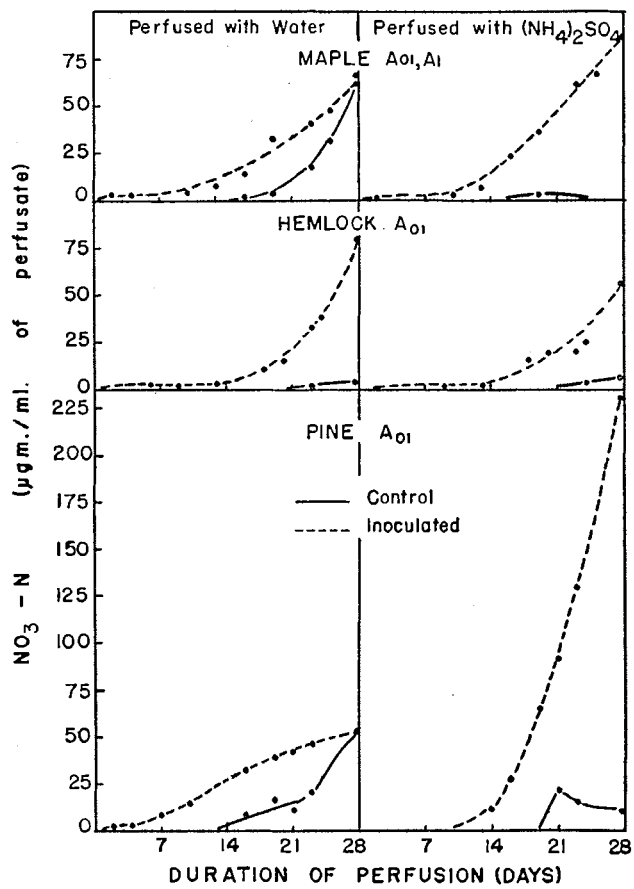

FIG. 1. Effect of inoculation with garden soil, and of adding ammonium-nitrogen, on nitrification in the organic layer of limed forest soil developed under three types of tree cover. No nitrification occurred when lime treatment was omitted.

them against the acid produced, when only the native soil nitrogen was nitrified; even more lime should have been used in those units perfused with ammonium sulphate solution, because of increased acid production.

The effects of inoculation and of adding ammonium-nitrogen on nitrification in the limed samples of the organic layers are illustrated in Fig. 1. The graphs show that inoculation started the nitrification process a week or two earlier than occurred in the uninoculated units. This advantage was maintained in the units containing added ammonium-nitrogen throughout the 28-day experimental period, but not in the units perfused with water. In two out of three of the latter units the initial advantage of inoculation had disappeared by the 28th day, suggesting that at this time ammonification of soil nitrogen had become the rate-limiting factor, rather than an inactive or small population of nitrifying bacteria. No reason can be given for the apparent inhibition of nitrification that occurred when the uninoculated organic layer was perfused with ammonium sulphate solution rather than water. This unexpected inhibition did not appear in any of the mineral samples of the three profiles. This is evident in Table V, which shows the nitrate-nitrogen produced in all the layers after 28 days. In general these results indicate that in the mineral layers, both inoculation and the addition of ammonium-nitrogen stimulated nitrification.

TABLE V

EFFECT OF INOCULATION WITH GARDEN SOIL ON NITRIFICATION IN LIMED SAMPLES OF FOREST SOIL WITH AND WITHOUT ADDED NITROGEN

Tree cover	Horizon	Nitrate-nitrogen formed in 28 days (μgm. per gm. air-dry soil)			
		Water perfusate		Ammonium sulphate perfusate	
		Control	Inoculated	Control	Inoculated
Maple	A_{01}, A_1	670	630	0	880
	B_2	150	135	180	1220
	B_{3g}	6	0	400	235
Hemlock	A_{01}	60	1620	120	1160
	A_1, A_2	85	135	90	255
	B_2	12	65	50	325
Pine	A_{01}	1080	1080	220	4660
	A_1, A_2	284	208	456	2070
	B_2	25	15	115	220

Discussion

This investigation of the way in which type of tree cover may influence the microorganisms in forest soil, was undertaken primarily as a survey, to discover whether a more intensive program would be justified, and if so, the most promising lines to follow. Nothing unusual was observed in the limited chemical studies made, and the association of a larger bacterial population in the organic layer under maple, and fungus populations under conifers, was similar to that found by others, both on this continent (3) and elsewhere (4). The occurrence of a predominant bacterial population beneath maple, in spite of the formation under maple of an acid mor commonly associated with conifers, certainly suggests that the nature of organic matter must be more important than pH. In this connection our findings in Ontario soil are in accord with results of other workers using soils from Idaho (18), and New York (3).

The nitrification studies showed that in the profile samples of this Ontario forest soil, lime applications were necessary before any nitrification occurred. Some improvement in nitrate production during laboratory studies involving lime treatment of forest soils has been reported by Powers and Bollen (12). The stimulation obtained from inoculation suggests that very few nitrifiers can be present normally. Furthermore, the amount of nitrate-nitrogen produced from native soil nitrogen, in the organic layers especially, indicates that appreciable quantities of nitrogen should be readily available, at least if the pH were adjusted by lime treatment. Also the increased rate of nitrate production that occurred when ammonium-nitrogen was added showed that the horizons in these profiles were capable, under ideal conditions, of a rate of nitrification that appeared to be well in excess of the ability of the organisms in the limed soil to release ammonia from the soil organic matter.

Since nitrification did not occur unless the samples were treated with lime, it seems that only organic and ammonium-nitrogen would normally be found in these profiles. It has also been observed that maples tend to be unthrifty in this region, but whether this is due to lack of nitrate-nitrogen, to the strong acid reaction of the soil, or to some other nutrient deficiency or environmental factor is not known.

While no attempt has been made to apply the findings to problems of forest regeneration, the subject of nitrogen transformations and nutrition of trees does appear worthy of further study. Actually, this problem is already receiving attention elsewhere, Gessel (5) having reported improved growth of Douglas fir following the use of nitrogen fertilizer in natural stands.

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